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PRINCIPAL INVESTIGATOR: Paola Muti, M.D.

CONTRACTING ORGANIZATION: State University of New York
Amherst, New York 14228-2567

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Paola Muti, M.D.

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Amherst, New York 14228-2567
Email - muti@buffalo.edu

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13. ABSTRACT ***(Maximum 200 Words)***

Experimental evidence shows that estrogens are related to prostate cancer risk – In addition recent data suggest that 16 α hydroxilated estrogen metabolites, biologically strong estrogens, are associated with cancer risk, while Z-hydroxyisted metabolites, with lower estrogenic activity, are weakly related to this disease. This study analyzes the association of prostate cancer with estrogen metabolism in a population-based study conducted in Erie and Niagara Counties – (the Protein Study) – Data in 81 prostate cancer cases and 318 control subjects showed that a higher ratio of 2 hydroxyestrone to 16 α hydroxyestrone uses associated with a reduced risk of prostate cancer.

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Introduction

Prostate cancer is the most common cancer among men in the United States (IARC, 1995). And the second most common in the European Community (IARC, 1995). The causes of prostate cancer, however, remain largely unknown, with age, race, and family histori being the only established risk factors (Nomura et al., 1997). The prostate gland has historically been considered the prototype of an androgen-dependent organ. However, there is evidence that estrogens may induce mitosis of prostatic epithelial cells in many species, including humans (Leav et al., 1978; Schulze et al., 1987). In humans, 16α hydroxyestrone and estriol are biologically significant estrogens, and their biologies can contribute to the overall expression of estrogenic action. Hydroxylation at the 16α position, one of the two major and mutually exclusive biotransformation pathways of estradiol, leads to estriol, a substance which is recognized as a biologically potent estrogen equivalent to estradiol (Clark et al., 1977). The other prominent pathway for metabolism is hydroxylation at C-2, producing 2-hydroxyestrone (Fishman J, 1963), a substance which has virtually no estrogenic activity (Gordon S.,1964; Martucci et al., 1977), except for some of the central activities of estradiol, such as the regulation of pituitary hormone release (Naftolin et al., 1975)

In 1979, Thomas Dao postulated that the way in which estrogens are metabolized might be important for cancer development (Dao T., 1979). The theory, known as "the unconventional estrogen hypothesis", proposed that the products of estrogen metabolism may be of etiological significance for hormone

related cancer. Several experimental, clinical and epidemiologic studies support this hypothesis (Schneider J et al., 1982; Clarck JK, et al., 1977; Fishman J. et al., 1980; Martucci et al., 1977). In *in-vitro* studies, 16α-hydroxylation has been shown to have strong biological estrogenic activity (Telang NT, et al., 1992; Suto A, et al. 1993) and genotoxic characteristics (Telang NT et al., 1992), while 2-hydroxylation metabolites had virtually no peripheral estrogenic effects (Suto A, et al., 1993). In mice, 16α-hydroxylation of estrone was associated with increased spontaneous incidence of tumors (Bradlow HL, et al.,1985).

When estrogen metabolism has been studied in relation to breast cancer risk in population-based studies, results supported the proposed hypothesis. In case-control studies an increase in estrone 16α-hydroxylation in breast cancer cases was observed compared with healthy controls, in particular in postmenopausal women (Osborn MP, et al., 1993; Ursin G, et al., 1997; Kabat GC, et al., 1997; Zheng W, et al., 1977). Two prospective studies conducted to investigate the role of estrogen metabolism as predictor of breast cancer concordantly found that study participants characterized by elevated 2-hydroxyestrone /16α-hydroxyestrone ratio had a 40% reduction in breast cancer risk compared with those in the lowest tertile (Meilahn EN et al., 1998; Muti P. et al., 2000).

The present report analyzes the association between prostate cancer and estrogen metabolism investigated in a case-control study. In particular, we tested the hypothesis that the pathway favoring 2-hydroxylation over 16α-hydroxylation may be associated with decrease in prostate cancer risk.

Body

Ninty-one prostate cancer cases and 318 control subjects participated in the study between 1999 and 2001. However, the active recruitment of participants started only after one year since it took a long time to get the approval of the study protocols from Institutional Review Boards of the five area hospitals. In the Statement of Work (Task 1), we estimated that we would only require 3 months to complete the approval process. This task, however, took almost a full year. We encountered some difficulties in obtaining approval, in particular, from one of the area hospitals. These difficulties however, have been resolved.

During the entire study period, we collaborated with a few physicians and have finalized the questions on risk factors for prostate cancer as well as preparing letters for both cases and controls and developing strategies for recruitment. In addition, we tried to expand the base of prostate cancer case recruitment by including all private practices in Erie and Niagara Counties. After six months of contacts and calls and visits, we had several offices that were collaborating with us.

We devoted massive attention, time and energy to recruit prostate cancer cases: a) we developed posters and brochures to be distributed to doctors' offices to support our recruitment efforts;

b) we also developed a staging form for the urogists to use in their private offices, memo pads and pens were distributed often along with a letter remiding them of the study and the need for their cancer case referral;

c) periodically, recent articles on prostate cancer etiology and treatment were sent to the area urologists again to remind them that the study was still on-going;

d) we met the HMO organizations and contacted them weekly to get support to our recruitment effort at the urologists's private offices;

e) in response to this, the Promedicus and the IPA sent letter to their participating urologists to help us in prostate cancer case enrollment. We also contacted a social organizations of prostate cancer cases such as US TOO and A FAMILY AFFAIR to inform their public about our research.

Although our efforts were equally spread all over the Erie and Niagara counties, most of our prostate cancer cases originated from three community hospitals in the Buffalo area: Buffalo General Hospital (BGH), Millard Fillmore Hospital (MFH), and the Department of Veterans Affairs Medical Center of Western New York Health Care System (VAMC). In particular, VAMC was remarkably efficient and open to our collaboration with a total of 64 prostate cancer cases recruited in the study there.

Prostate cancer patient recruitment: All patients, 65 to 85 years old, with newly diagnosed, cytologically and/or histologically confirmed prostate cancer have been considered for the study. All cases have been recruited in the study *before starting any cancer treatment.* After obtaining names of cases with newly

diagnosed prostate cancer, we mailed a request to the physician for permission to interview the patient. Once the approval was received, we sent a letter to the patient inviting them to participate. A written consent was obtained from the patient for both the blood draw and for the interview before the actual process took place

Eligibility criteria Since the major focus of the study was the relation of estrogen metabolism with prostate cancer risk, patients on hormonal treatment (current or in the six months prior the diagnosis), or affected with metabolic diseases affecting endocrine profile (i.e., hypogonadism, hyperadrenalism) were excluded. Those affected with chronic or acute liver diseases also were excluded because of their potential influence on the endocrine pattern. Patients with a previous history of cancer (except of skin cancer) have been excluded as well.

During the active phase of study recruitment (December 1999- April 2001), 146 prostate cancer cases have been identified. Of these 146, 108 met eligibility criteria, and were approved by the physicians at VAMC and invited to join the PROMEN study. Of these 108, 17 refused to participate. Thus, 74% (108/146) of the subjects were potential participants in the study, while the actual participation rate was 84% (91/108).

Prostate control recruitment: Control have been men, 65 to 85 years of age, selected from the rolls of the Health Care Finance Administration and matched

with prostate cancer cases on area of residence (first three digits of the zip code).

Eligibility criteria Eligibility criteria for controls was the same as for cases. We excluded men on hormonal treatment (current or in the six months prior to the contact), or affected with metabolic or endocrine diseases. Participants with a previous history of cancer (except of skin cancer) have been excluded as well.

During the same fifteen months of the study, 489 eligible controls were contacted. 84 of these potential candidates were deceased and 52 were too ill to participate (136/489, 27% of the sample). 318 of the remaining 353 subjects (90%) were enrolled and interviewed.

Since there is high prevalence of latent prostate carcinoma in men over age 50 (Breslow et al., 1977; Bostwick et al., 1992), we determined Prostate-Specific Antigen (PSA) in all blood samples obtained from controls.Controls with a PSA value higher than 4ng/ml were excluded from the control group according to the criterion proposed by the American Cancer Society Prostate Cancer Detection Project (Babaian et al., 1992) until the completion of diagnostic procedures to determine their true case-control status.

We have been able to identify 8 prostate cancer cases as a result of the PSA determinations that were done in controls. The PSA results were sent to the

participant's primary physician along with a letter to draw attention to the fact that it was outside of the normal range. The primary physician then referred the participant to an urologist where a biopsy was done to confirm prostate cancer. In our records, we modified the case-control status after the confirmation of prostate cancer diagnosis.

During the study period, procedures have been finalized for the ongoing maintenance of the biological specimen bank, processing of samples for immediate determinations and for storage, tracking of samples and mapping of the freezer as outlined in the Statement of Work (Task 2). For standardization purposes, morning spot urine was collected and fasting samples (57ml) of blood was drawn during the same time intervall of the day (7:00AM – 9:00AM) from all participants. The time at specimens collection was recorded.

In accordance with the Statement of Work (Task 2), preparations for the data entry of the interview, maintenance of files from the computer-assisted interview, and entry of data from the sections of the interview completed by hand by the participant were developed during the recruitment phase of the study. The database for the prostate cancer study is written in Microsoft Access and contains information on all participants. Each form that a study participant filled out has a computer equivalent. Each of these databases is housed in the same location for security, backup, and data analysis purposes. These programs were written in Microsoft Access as well. Data validation, analysis, and compatibility

(for integration with SPSS) have been written in Visual Basic. Training has also been provided for the identification and staging of prostate cancer cases in area hospitals as well as the private physician offices.

Hormonal derminations in prostate cancer cases and control subjects (Tasks 4 to 5)

Stored urinary samples from prostate cancer cases and related controls were handled identically and randomly located across the laboratory runs. All laboratory personnel were blinded with regard to case-control status.

Analyses of 2-hydroxyestrone (2OHE1) and 16α-hydroxyestrone (16α-OHE1) were performed using a competitive solid-phase enzyme immunoassay (IMMUNA CARE Corporation, Bethlehem, PA). The urinary forms of these estrogen metabolites are found as glucuronide conjugates and require the removal of the sugar moiety before recognition by the monoclonal antibodies. A mixture of β-glucuronidase and arylsulphatase (glusulase from *H. Pomatia*, Sigma Chemical Co., St. Louis, MO) was used for this purpose. The enzyme digest was then neutralized. Assay incubation time was 3 hours at room temperature. The assay was read kinetically using a Ceres 900 HDI plate reader (Biotek Instruments, Winooski, VT) and the data were reduced using Kineticalc EIA Application software (Bio-Tek® Instruments). Both assays have been shown to demonstrate 100% recovery of metabolites with serial dilution and "spiking" of exogenous estrogens into urine samples. The EIA kits have been evaluated for validity and reproducibility and the values for each metabolite were compared

with values obtained by gas chromatography-mass spectrometry (Bradlow HL. Et al., 1998; Klug TL, et al., 1994; Sepkovic DW et al., 1994). As a measure of reproducibility, control samples were included and their values had to fall within two standard deviations from the mean of a continuous Levy-Jennings control plot. In addition, 10% duplicates were included with each batch of samples to determine reproducibility. Intra -assay coefficienst of variation for 2-OHE1 and 16-αOHE1 were 3.6% and 3.8%, respectively. Interassay coefficients of variantion were 5.9% and 10.2%, respectively.

Statistical Analysis (Task 6)

2-hydroxyestrone and 16α-hydroxyestrone urinary levels were standardized by the total creatinine. We used conditional logistic regression to obtain the odds ratios of prostate cancer in relation to estrogen metabolites and their ratio (Breslow NE, Day NE, 1980). The independent variables of interest were 2-OHE1, 16α-OHE1, and the ratio of 2-OHE1 and 16α-OHE1 by tertiles of urine concentration. We based the cutoff points for each tertile on the distribution of the estrogen metabolites in controls. In the present analysis on estrogen metabolism, we included 70 prostate cancer cases since 21 participants refused to donate biological specimens and accepted only to answer to the questionnaires. 318 controls were available for the present analysis.

We identified age, weight, waist-to-hip ratio, as potential covariates according to their potential biologic relevance and logistic regression was used to control for these covariates. Since the information on all covariates was missing for part of the prostate cancer cases and control subjects, a total number of 61

prostate cancer cases and 235 controls were then considered in the analysis considering more complex models. In the initial regression model, we examined all variables. We evaluated each covariate for confounding by removing each from the fully adjusted model. Age, weight, and waist-to-hip ratio, did not substantially modify the results. None of the potential covariates was a confounder of the association between prostate cancer and estrogen metabolites and their ratio. Nevertheless, we included them in further analysis to provide fully adjusted estimates for comparison with those reported in the published literature, in particular with the previous studies on hormones and prostate cancer risk.

Study results (task 7).

Characteristics of the study population are reported in Table 1.

Prostate cancer cases were more likely to be slightly younger and to have higher weight, and a higher waist-to-hip ratio and a lower education. In a descriptive analysis reported in Table 2 conducted on control subjects, urinary levels of estrogen metabolites did not significantly differ by age strata although both 2-OHE1 and 16-αOHE1 were higher in older age in both prostate cancer cases and controls while the 2-OHE1/ 16-αOHE1 ratio was higher in younger subjects. African Americans showed to have a higher levels of estrogen metabolites and a higher ratio, although the difference did not reach the statistical significance due to the low number of African American study participants. Estrogen metabolites differ by waist-to-hip ratio tertiles with the lowest levels of estrogen metabolites and their ratio being observed in the

highest tertile of abdominal fat. Lighter subjects showed to have significant higher levels of 2-OHE1 and significant higher level of 2-OHE1/ 16-αOHE1 ratio. Current smokers showed to have a higher levels of estrogen metabolites, and the lowest ratio, in comparison with never and former smokers. Finally, education, as an index of social and economic status and thus of life-style pattern, showed to have an effect on the estrogen metabolism. Participants classified in the lowest education level had the highest levels of both 2OHE1 and 16- αOHE1 and the lowest ratio .

Table 3 reports data on prostate cancer risk in relation to tertile of distribution of the estrogen metabolites, their ratio, and their 95% coefficients of variation. There was a light protective effect for the highest tertile of the 2-OHE1, however the confidence intervals included the unity. Conversely, there was a light risk effect in the highest tertile of 16α-OHE1 and again the confidence intervals included the unity .

The increase in 2-OHE1/16α-OHE1 ratio was associated with a reduction in odds ratios for prostate cancer across tertiles: the highest tertiles of 2-OHE1/16α-OHE1 ratio had the lowest risk estimate, even after adjustment for covariates.

Key Research accomplishments.

The present research has found that:

- Estrogen metabolites can be found and measured in men;
- Estrogen metabolism is affected by age, abdominal adiposity, weight, smoking status, and education as an index of social and economic status;
- Estrogen metabolism may be implicated in prostate cancer development;
- Estrogen metabolism may become a potential tool for preventive strategies against the development of prostate cancer and for its therapeutic management.

Reportable Outcomes

The present data have to be considered preliminary results since the laboratory determinations have been completed on May 15th. However, we included in the present report data from a corollary preliminary study conducted in the context of the PROMEN study in collaboration with the Johns Hopkins University, Baltimore, MD. The study focused on the relation between human papilloma virus infection and risk of prostate cancer. Preliminary results of that study are included in the appendix in the form of a poster for a conference communication. The data will be presented by Dr. Sharita Womak, a NCI postdoctoral fellow mentored by Dr. Muti at Professional Development Workshop, sponsored by the Comprehensive Minority Biomedical Branch (CMBB),Office of Centers,Training,Resources (OCTR), Office of Deputy Director for Extramural Science (ODDES), and National Cancer Institute (NCI);June 4-6, 2001.

The final aim of the HPV determination is to evaluate potential biological interaction between viral infection and estrogen metabolism in the development of prostate cancer.

Conclusions.

The main finding of the present study was that estrogen metabolism pathway favoring 2-hydroxylation over 16α-hydroxylation is associated with a reduced risk of prostate cancer risk. This is the first time that estrogen metabolism has been studied in relation to prostate cancer and the first observation supporting the potential protective role of estrogen metabolites characterized by low biological activity in prostate cancer development.

Analytical epidemiological studies on effects of estrogens in relation to prostate cancer risk, in particular serum estrone and estradiol have provided conflicting results. Serum estrone levels in patients with prostatic cancer have been reported to be higher than (Jackson et al., in African American,1980; Ahluwalia et al., 1981; Hill et al., 1982; Zumoff et al., 1982), lower than (Drafta et al., 1982) and similar to (Bartsch et al.,1977a; Bartsch et al.,1977b; Jackson et al.,in Nigerians,1980;Nomura et al., 1988; Hsing et al., 1989; Barrett-Connor et al., 1990; Signorello et al., 1997) those in healthy controls. Serum estradiol showed similar conflicting results (Harper et al., 1976; Bartsch et al.,1977a; Bartsch et al.,1977b; Hammond et al., 1978; Ahluwalia et al., 1981; Hill et al., 1982; Hoisaeter et al., 1982; Meikle et al., 1982; Ranikko et al., 1983; Hulka et al., 1987; Hsing et al., 1989;Meikle et al., 1989; Andersson et al., 1993; Gann et al., 1996; Signorello et al., 1997 Drafta et al., 1982).

The inconsistency of results may be due to chance, low statistical power (low number of cases and controls), different strategy of control selection and different methodology in specimen collection (i.e., different control of some

sources of hormone variability as circadian rhythm or different storage conditions). It may also be that the relevant measure was not the serum level of estrone and estradiol but the estrogen metabolism.

Plasma concentrations of unconjugated estriol and 16α hydroxyestrone are low relative to estrone and estradiol (Fishman et al., 1976; Longcope et al., 1977) but their biological impact may be significant because of their lack of affinity for SHBG (sex hormone binding globulin) (Fishman et al., 1980). Considering that 16α hydroxyestrone is virtually unsequestered by SHBG, it may be that its biological effectiveness could equal or even exceed that of estradiol, even though the concentration of the latter in plasma is several-fold greater. In circumstances of comparable hormone secretion, therefore, estradiol metabolism shifted in the 16α hydroxylation direction could produce a hyperestrogenic milieu, while a predominance of 2-hydroxylation could produce hypoestrogenic conditions. 16α hydroxyestrone has been found to be elevated in strains of mice susceptible to breast cancer (Bradlow,1985). In humans, estrogen metabolism has been primarily studied in women in relation to breast cancer risk. There is some evidence that 16α hydroxyestrone is elevated in women with breast cancer (Bradlow, 86; Fishman et al., 1984, Kabat et al., 1997, Muti. 2000).

There are a number of potential explanations for these findings, including potential effects of the neoplastic tissue on estrogen metabolism. Another potential source of bias in our study may be related to the preferential participation of prostate cancer cases from the Department of Veterans Affairs Medical Center of Western New York Health Care System (VAMC) while control

subjects were selected from the general population. Thus, differences in social and economic class and lifestyle may explain the present results. In order to limit the potential effects of social and economic and lifestyle bias, the control subjects were frequency-matched on area of residence (neighborhood controls) and randomly selected every two weeks from the general population of Erie and Niagara Counties. In the present study urinary estrogen metabolite levels were determined controlling for several sources of hormone variability by both inclusion criteria and highly standardized conditions at blood drawing. Cases were drawn before cancer treatment was begun, and control subjects were evaluated for potential presence of latent prostate cancer by serum analysis for prostate-specific antigen. Cases and controls on current or recent (six months prior the participation in the study) local or systemic hormonal therapy, and those affected with endocrine diseases, acute or chronic liver diseases were excluded to reduce, at least in part, some of the sources of hormone variability potentially influencing study results. Extreme cure was taken in order to control for hormone circadian variation. All hormone determinations for all cases and controls were performed at the end of the recruitment phase of the study, during the last month of the study period to reduce interassay variability. The laboratory assaying estrogen metabolites was blinded to case-control status.

In conclusion, this study supports the hypothesis that there is difference in the way estrogens are metabolized between patients affected with prostate cancer and control subjects. Further studies are needed to corroborate these findings and to offer a new perspective on hormone involvement in prostate

cancer development. This, in turn, will allow to improve prevention and/or treatment.

Table 1. Characteristics of the Study Participants

	Cases		Controls	
	n	%	n	%
Age (years)				
45-64	12	19.7	21	8.9
65-74	36	59.0	142	60.4
75-85	13	21.3	72	30.6
total	61	100.0	235	100.0
Race				
Whites	44	72.1	213	90.6
African Americans	17	27.9	22	9.4
Waist-to-hip ratio (tertiles)				
1st	19	31.1	79	33.6
2nd	16	26.2	83	35.3
3rd	26	42.6	73	31.1
Weight (tertiles)				
1st	17	27.9	82	34.9
2nd	21	34.4	76	32.3
3rd	23	37.7	77	32.8
Smoking status				
Never	20	32.8	79	33.6
Current	6	9.8	15	6.4
Former	35	57.4	141	60.0
Education				
Less than high school	20	32.8	37	15.7
High school	18	29.5	81	34.5
College	23	37.7	117	49.8

Table 2. Levels of Estrogen Metabolites among Participants without Prostate Cancer

		2-hydroxyestrone (2-OHE1)		16α- hydroxyestrone (16α-OHE1)		2-OHE1/16α-OHE1 ratio	
	n	Mean	SD	Mean	SD	Mean	SD
Age (yrs)							
45-64	21	5.40	2.24	4.42	2.22	1.35	0.60
65-74	142	5.67	2.77	4.33	1.74	1.36	0.53
75-85	72	5.54	2.34	4.52	1.83	1.29	0.47
P		*0.88*		*0.77*		*0.64*	
Race							
White	213	5.56	2.48	4.38	1.70	1.33	0.52
Black	22	6.11	3.53	4.53	2.71	1.46	0.58
P		*0.34*		*0.72*		*0.27*	
W/H ratio							
1st	79	6.00	2.52	4.33	1.63	1.46	0.58
2nd	83	6.00	2.64	4.61	2.12	1.38	0.45
3rd	73	4.72	2.42	4.22	1.59	1.67	0.49
P		*0.002*		*0.39*		*0.002*	
Weight							
1st	82	6.19	2.43	4.53	1.68	1.44	0.54
2nd	76	5.55	2.75	4.43	2.05	1.32	0.48
3rd	77	5.04	2.49	4.21	1.68	1.25	0.54
P		*0.02*		*0.53*		*0.07*	
Never	79	5.45	2.40	4.23	1.53	1.35	0.53
Current	15	5.85	2.23	5.63	2.53	1.15	0.51
Former	141	5.67	2.74	4.36	1.83	1.36	0.52
P		*0.77*		*0.02*		*0.36*	
Education							
<High sc	37	5.80	3.12	4.64	2.27	1.32	0.48
High sc	81	5.64	2.20	4.59	1.82	1.30	0.50
College	117	5.52	2.68	4.18	1.61	1.37	0.55
P		*0.85*		*0.20*		*0.61*	

N, sample size. SD, standard deviation. P, p value for test in mean differences.
W/H ratio, waist-to-hip ratio (tertile). Sc, School.

Table 3. Prostrate Cancer Risk by Tertiles of Estrogen Metabolite Levels and Their Ratio

	n (Cases/Controls)	Crude OR (95% CI)	Adjusted OR * (95% CI)
2-hydroxyestrone (2-OHE1)			
1st	22/76	1.00 (reference)	1.00 (reference)
2nd	24/75	1.11 (0.57-2.14)	1.11 (0.54-2.26)
3rd	15/84	0.62 (0.30-1.28)	0.61 (0.28-1.33)
16α- hydroxyestrone (16α-OHE1)			
1st	19/79	1.00 (reference)	1.00 (reference)
2nd	15/84	0.74 (0.35-1.56)	0.90 (0.41-1.96)
3rd	27/72	1.56 (0.80-1.04)	1.73 (0.84-3.55)
2-OHE1/16α-OHE1 ratio			
1st	25/73	1.00 (reference)	1.00 (reference)
2nd	22/77	0.83 (0.43-1.61)	0.80 (0.40-1.59)
3rd	14/85	0.48 (0.23-0.99)	0.46 (0.21-0.99)

OR, odds ratio. 95% CI, 95% confidence interval.

* Adjusted for age, race, education, smoking status, and waist-hip-ratio using logistic regression.

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Appendices

Poster on HPV and prostate cancer risk.

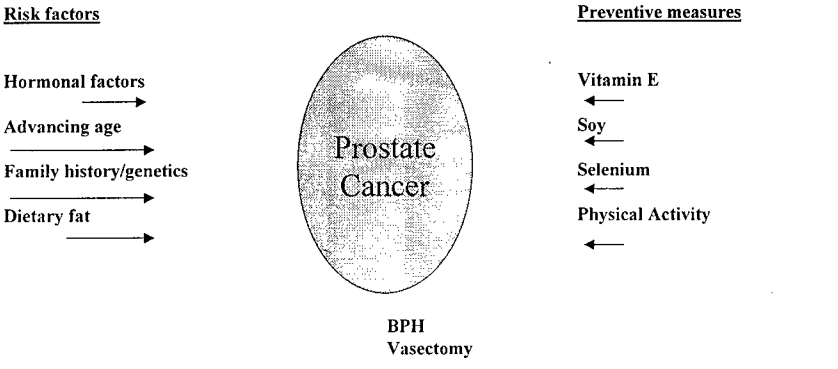
Human papillomavirus seropositivity in PROMEN case and control study participants

Preliminary Results

Background

- Prostate Cancer is leading cancer diagnosed among men (*SEER, 1988-92*)
- 2nd leading cause of male cancer deaths in the country (*Parkin et al, 1997*)
- In 2000, 180,400 men would be diagnosed and 37,000 deaths (*ACS facts and figures, 1999*)
- Mortality rates are more than twice as high as rates in white men (*SEER 1988-92*)
- Factors suggested to play a role: age, weight, high fat diet, vasectomy, physical activity, and *infectious agents* (*Ross RK and Schottenfield D, 1996*)
- HPV has been reported from prostate tissues in several recent investigations (*Cuzick, 1995*)

Common Risk Factors and Preventive Factors for Prostate Cancer



Gallagher et al, CMAJ 159(7) 1998; updated

Role of Sexual Behavior in Prostate Cancer Etiology

- Key et al., 1995 (systematic review)
  - --- sexual behavior emerged as one of the stronger and more consistently identified risk factors
  - --- increased RR for early intercourse and for multiple sexual partners
  - --- highest RR was for a history of sexually transmitted disease (RR=1.86; 95% CI, 1.43-2.42)
  - --- recall and selection bias

- Two cohort studies
  - --- (Nilsen TL et al, 2000 and Hayes RB et al, 2000)
  - --- found that men who were divorced or separated ↑
  - --- syphilis, gonorrhea, sex with prostitutes and unprotected sexual intercourse were indicators of contact with a STI ↑

HPV infection of the Prostate and Prostate Cancer Risk

- HPV has been detected in prostate tissues in several recent investigations
- Polymerase chain reaction (PCR) and other methods
- Case-control studies have provided conflicting results

Table 1

Summary of Studies Investigating HPV in the Prostate Specimen

			Subjects		
Reference	**HPV detection**		**No.**	**Type**	**HPV %**
McNicol 1990	Southern blot for HPV-16 and -18	4 SPP	PCa cases 12	75% BPH control	33%
McNicol 1990	E6 PCR HPV-16, -18	4	PCa cases 5	100% BPH controls normal autop.	93% 20%
Masood 1991	ISH for HPV-6,11, 1618,31,33,35	20	PCa cases 20	none BPH controls	none
MCNicol 1991	E6 PCR HPV-16, 18	27	PCa cases 56	52% BPH controls	63%
Anwar 1992	E6 PCR HPV-16, 18,33	68	PCa cases 10 10	41% BPH controls normal autop.	none None
Effort 1992	Differential E6 PCR HPV-16 and 18	30	Pac cases 8	none Cervical canc.	38%
Dodd 1993	Reverse transcription; PCR E6/E7 MRNA of HPV-16		7 10	PCa cases BPH controls	43% 50%
Tu 1994	L1 consensus primer PCR	43	PCa cases 17 1	2% metastases normal	6% none
Moyret-Lalle 1995	E6 PCR HPV-16; and 18	17	PCa cases 22	53% BPH controls	32%
Wideroff 1996	L1 consensus primer PCR, E6 PCR for SPP HPV-6,11,16,18,31, 33,and 45	56 42	PCa cases BPH controls	L1 13% L1 10%	E6 0% E6 0%
Suzuki 1996	L1 consensus primer	51	PCa cases	16%	
Strickler 1998	L1 consensus primer And E6 PCR	63	PCa cases 61	none BPH controls	none
	Serum antibodies HPV -16 and -11		63 144	PCa cases BPH controls	no difference

HPV infection of the Prostate and Prostate Cancer Risk

- 2 Prospective Studies (Dillner et al, 1998 and Hisada et al, 2000) increased prostate cancer risk among subjects with HPV 16/18 antibody compared with those w/o the antibody
  - » 2.4 to 2.6 fold risk ($p < 0.005$)
  - » OR= 2.7; 95% (CI, 0.9-7.9)

Specific Aims

1.To determine the HPV prevalence of serum antibodies to HPV 16, 18, and 31 in stored serum specimens collected from the first 79 prostate cancer cases and 280 control subjects in the ongoing PROMEN study.

2. To determine the strength of the association of HPV seropositivity in PROMEN case and control study participants.

Elisa Assay

- HPV 16, 18, and 31 Virus-like particles (VLP) prepared from insect cells (expressing the L1 and L2 viral capsid protein)
- VLP's coat wells of 96-well mirotiter plate
- control wells coated with PBS
- plates washed 5 times
- add test serum to each antigen
- incubated 2 hrs at 37^0C
- plates washed 5 times
- VLP reaction detected with horseradish peroxidase conjugated Protein G
- incubate 30min
- hydrogen peroxide solution (incubate 30 min)

PROMEN Study

PROstate cancer and Metabolism of EstrogeNs

- An ongoing study case-control investigation of the of prostate cancer and two estrogen hormones.
- This study is being conducted in the greater Buffalo, New York area.
- Funded by the U.S Army Prostate Cancer Research Program
- Includes men, 49-85 with incident, primary, pathologically confirmed prostate cancer
  - diagnosed at the Department of Veterans Affairs Medical Center of Western New York (VAMC),
    - ECMC, Buffalo General, Millard Fillmore and RPCI

PROMEN Study
continue....

•Data collected:

--***physical measurements*** :weight, height, waist to hip, etc

--***questionnaire***-- sexual behavior across different age periods, expressed as number of partners;age at first intercourse;

lifetime alcohol intake history, reproductive history, diet history, medical history, residential history, physical activity and vasectomy

--***clinical specimens***--blood and urine specimens

--questionnaires and measurements repeated in 51 controls over a one year period **(test reproducibility)**

PROMEN Study
continue....

• Cases:

men attending annual physical exam at VAMC and other clinics in Buffalo
49-85 years old, with newly diagnosed histologically confirmed prostate cancer prior to cancer treatment

to assure more accurate case definition, only clinical apparent prostate cancer cases (stage B and over) were included

participation rate was 84%

PROMEN Study

continue....

- Controls:

  men 49-85 years old
  selected from driver's licenses list of Erie and Niagara county residence;
  controls age 65 and over are randomly selected from the rolls
  of the Health Care Finance Administration
  frequency matched to cases on age, race, and area of current residence

  participation rate 72%

PROMEN Study

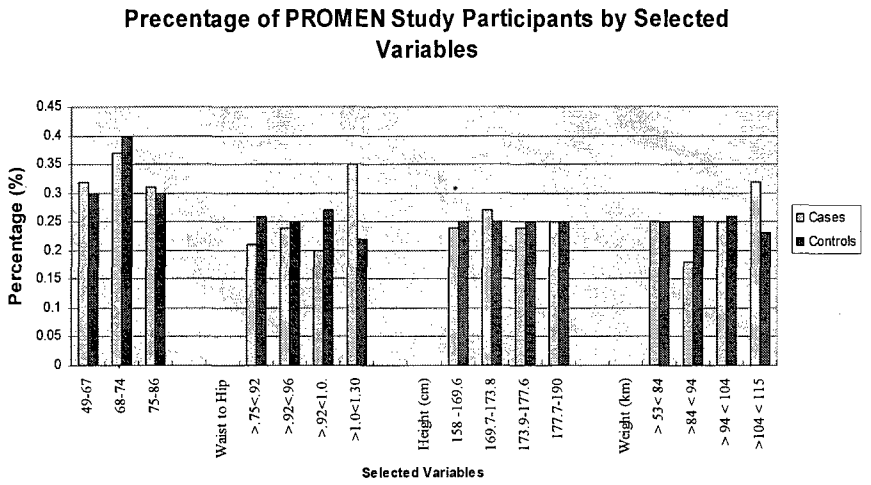
continue....

- Exclusion criteria:

  - cases: on hormonal treatment or with metabolic/endocrine diseases
    affected with chronic or acute liver disease
    with previous history of cancer

  - controls: on hormonal treatment or with metabolic disease or endocrine diseases
    PSA value higher than 4ng/ml (to exclude potential latent carcinoma)

RESULTS: Figure 1



RESULTS: Table 2

Reliability Coefficient Selected Variables

Waist to Hip	0.82
Height (cm)	0.96
Weight (km)	0.90

Results: Table 3
Sexual Behavior and Reproductive Variables of PROMEN

Selected Variables	Cases Mean (SD)	Controls Mean (SD)	Reliability coefficient
Age first intercourse	17.9 (4.20)	19.5 (4.02)*	0.97
# sex partners at 20-30	3.8 (4.4)	3.6 (4.7)	.70
# sex partners at 31-40	3.0 (4.6)	1.8 (4.6)*	.87
# sex partners at 41-50	2.8 (4.3)	1.5 (3.8)*	.49
# sex partners at 51-60	2.1 (3.3)	1.3 (3.1)	.91
# sex partners at 61-70	1.8 (3.7)	1.1 (2.6)	.87
# subjects with vasectomy	7 (7%) †	19 (6.6%) †	1.00**

*t-test for unpaired data (P<0.05); † prevalence in percentage; ** percent agreement

Results: Table 4
Adjusted Odds Ratio (OR) of PROMEN Study Participants for HPV 16, 18, and 31 seropositivity

Infectious agent	**Percent Positive**		**Crude OR (95% CI)**	**OR adjusted for age, race and BMI**	**OR adjusted for age, race, WHR**
	Cases	Controls			
HPV 16	3	.4	7.3 (.65-81.0)	2.7 (.15-45.1)	2.8 (.23-34.4)
HPV 18	11	11	1.01 (.49-2.4)	1.1 (.48-2.6)	0.9 (.37-2.1)
HPV 31	4	.7	5.5 (.90-33.4)	4.5 (.55-37.2)	6.9 (1.1-44.6)
HPV 16, 18, 31	20	10	1.5 (.74-3.1)	1.4 (.63-3.1)	1.3 (.62-2.8)

Conclusion

1) ↑ WHR and weight are related to increased risk
   » *Cancer Epid., Biomark. & Prev. (Hsing et al, 2000)*

2) Increased number of sexual partners over decades and earlier age at first intercourse were shown for cases (..recall bias may exist)

3) Serology date indicate a potential association between HPV infections and prostate cancer risk, however estimates always included unity
   » small sample size or
   » large variability of the serological method

Future Studies

1) Strength of association of prostate cancer using two more sensitive HPV detection assay/method performed in urine and compare results with current serology data for HPV 16, 18, 31

   »L1 consensus primer-based polyerase chain reaction (PCR)
   »Hybrid Capture II probe B)

2) To evaluate potential variants in HPV 16, 18, and 31 in relation to prostate cancer risk

3) Immunologic characteristics in relation to HPV and cancer risk

Limitations

1) ***Serological measurements of HPV is consistently reported as an insensitive method compared to other assays/method***
   - » low titer
   - » not detected in a proportion of subjects previously or currently HPV positive

2) **Cause-Effect relationship can not be derived from case-control study**

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Dr. KV Shah

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 26 Nov 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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